Septicaemia due to a *Proteus* infection in a Humboldt penguin (*Spheniscus humboldti*)

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**Abstract**: A 2-year-old male Humboldt penguin (*Spheniscus humboldti*) died after a very brief period of illness at a zoo aquarium; the penguin showed sudden depression, anorexia, dyspnoea, and had recurrent melena a day prior to death. The gross examination revealed an extensive bilious effusion in the abdominal cavity due to a gallbladder rupture. Moreover, abscess formation, purulent exudate, severe congestion, and haemorrhages were observed in the trachea and parenchymal organs such as the kidneys and the lungs. A histopathological examination revealed a fibrin deposition with a severe haemorrhage and secondary infiltration of chronic-active inflammatory cells in the parabronchi, atria, and air capillaries and blood vessels of the lungs as well as in most of the parenchymal organs. Moreover, Gram-negative bacilli were found in the lumen of the gastrointestinal tracts including the small and large intestines accompanied by severe epithelial necrosis and the capsule of the liver. Especially, bile pigments were microscopically observed in the whole liver, which indicated a gallbladder rupture. Samples collected from the trachea, lungs, and blood were cultured on a blood agar, and the pure colonies of *Proteus* genus were isolated. *Proteus mirabilis, P. penneri, P. vulgaris, and P. cibarius* were identified with polymerase chain reaction (PCR). As a result, the diagnosis was confirmed as *Proteus* septicaemia. To our knowledge, this is the first report of concomitant infection by different *Proteus* species that eventually resulted in septicaemia in a Humboldt penguin, and it will provide valuable information for zoo veterinarians for its diagnosis as well, since Humboldt penguins are the most widely found penguins in zoos and *Proteus* septicaemia in the penguins has, to the best of our knowledge, not been reported as yet.

**Keywords**: aquatic animals; aves; haemorrhage; parenchymal organs; bilious effusion; sepsis; histopathology

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Proteus, a genus of the family Enterobacteriaceae, is an opportunistic pathogen that causes severe septicaemia in humans and animals with an underlying disease or infection (Sah et al. 1983; Rozalski et al. 1997). The most common infection-causing species are P. mirabilis, P. vulgaris, and P. penneri. These species cause bloodstream infections (BSI), wound infections, urinary tract infections (UTI), asymptomatic bacteriuria, meningoencephalitis, and respiratory system infections (Kim et al. 2003; Stock 2003; Endimiani et al. 2005). Proteus cibarius has recently been reported to have resistance to all β-lactam antibiotics and causing wound infections in humans, but its clinical significance has not been reported in animals (Adesina et al. 2018).

In humans, several cases of Proteus septicaemia are reported annually; therefore, its significance is duly recognised (O’Hara et al. 2000; Chen et al. 2012; Kwiecinska-Pirog et al. 2018). However, this is not the case in animals, although some cases of Proteus infections have been reported in the canine and feline species (Lees 1996; Ling et al. 2001); moreover, general information, including symptoms, histopathological findings, prognosis, and preventive measures, still remain ambiguous in veterinary medicine. Particularly, cases of Proteus septicaemia are extremely rare with no reports available to us in penguins until date. Therefore, Proteus was not a primary pathogen to be commonly diagnosed in avian septicaemia. In that regard, a detailed histopathological analysis on the septicaemia caused by the Proteus species in this animal may contribute to diagnosing Proteus septicaemia in avian species, especially in penguins including S. humboldti. For these reasons, we are reporting this apparently first known case of lethal Proteus septicaemia in a Humboldt penguin.

Case Description

A 2-year-old, male Humboldt penguin, which was transferred from a local zoo aquarium, was presented for necropsy. The penguin was in moult (Figure 1A) and expressed an aggressive behaviour towards the other penguins, so it was introduced to a new rearing aquarium. A week after, the penguin showed sudden depression, anorexia, dyspnoea, and had recurrent massive melena the day before death. The penguin otherwise had a clean medical history. A complete necropsy was performed 12 h after death. Overall, no ex-
ternal signs of haemorrhages or trauma were evident (Figure 1A). However, the whole abdominal cavity contained large quantities of a bilious dark greenish effusion resulting from a ruptured gallbladder (Figure 1B). The abdominal viscera were stained with the dark greenish effusion (Figure 1C and 1D). The kidneys were severely oedematous and congested with abscesses on the cortex of both the kidneys (Figure 1C). The liver was slightly swollen and severely congested (Figure 1D). The mucosa of the gastrointestinal tracts was also oedematous and hyperaemic. Marked consolidation, thrombi, oedema, haemorrhages, and congestions were diffusely identified in both lungs (Figure 1E). Along with the kidneys, a purulent and haemorrhagic exudate was collected at the caudoventral portions of the lungs and at the tracheal carina (Figure 1F). Bilateral congestion and hyperaemia of the brain were also noted.

For the microscopic examination, the collected tissue samples were fixed in a 10% neutral buffered formalin, routinely processed, paraffin-embedded,
samples were inoculated onto a blood agar media (KisanBio, Seoul, Republic of Korea) for routine aerobic bacterial isolation. Moreover, an AnaeroBag kit (KisanBio, Seoul, Republic of Korea) was used for the anaerobe and microaerophile culture. All samples were inoculated onto a blood agar media (KisanBio, Seoul, Republic of Korea) for routine aerobic bacterial isolation. Moreover, an AnaeroBag kit (KisanBio, Seoul, Republic of Korea) was used for the anaerobe and microaerophile culture. All samples were inoculated onto a blood agar media (KisanBio, Seoul, Republic of Korea) for routine aerobic bacterial isolation. Moreover, an AnaeroBag kit (KisanBio, Seoul, Republic of Korea) was used for the anaerobe and microaerophile culture. All samples were inoculated onto a blood agar media (KisanBio, Seoul, Republic of Korea) for routine aerobic bacterial isolation. Moreover, an AnaeroBag kit (KisanBio, Seoul, Republic of Korea) was used for the anaerobe and microaerophile culture. 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the media under aerobic, anaerobic, and microaerophilic culture conditions were incubated at 37 °C for 24 hours. After that, the isolated colonies were once again inoculated onto a 5% sheep blood agar (Mbcell, Seoul, Republic of Korea), incubated at 37 °C for 24 h for further identification, and polymerase chain reaction (PCR) investigations were performed. The 16S rRNA of these isolated bacterial colonies in the culture media were identified by using the universal primers 27F (5’-AGA GTT TGA TCC TGG CTC AG-3’) and 1492R (5’-GGT TAC CTT GTT ACG ACT T-3’). As a result, the Proteus species was identified in the samples, including the blood sample (Table 1).

<table>
<thead>
<tr>
<th>No.</th>
<th>Abdominal cavity</th>
<th>Blood</th>
<th>Trachea</th>
<th>Lung</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Proteus cibarius</td>
<td>Proteus cibarius</td>
<td>Proteus cibarius</td>
<td>Proteus cibarius</td>
<td>Escherichia fergusonii</td>
</tr>
<tr>
<td>2</td>
<td>Proteus mirabilis</td>
<td>Proteus mirabilis</td>
<td>Proteus vulgaris</td>
<td>Proteus vulgaris</td>
<td>Escherichia fergusonii</td>
</tr>
<tr>
<td>3</td>
<td>Proteus vulgaris</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tr>
</tbody>
</table>

The PCR-based bacterial identification of the samples collected from the abdominal cavity, kidneys, trachea, lungs, and blood. The Proteus species and E. fergusonii were detected in the whole liver section, indicating a gallbladder rupture. Moreover, rod-shaped Gram-negative bacteria were found in the capsule surface of the liver. Additionally, the intestines were characterised by fusion and necrosis of the villi, with numerous Gram-negative bacilli in the lumen. The lungs also showed severe haemorrhages in the parabronchi, atria, air capillaries, and interparabronchial septa and diffuse microvascular coagulation intermingled with the inflammatory cells without an indication of a primary underlying vasculopathy disease. These findings strongly suggest that this bird succumbed to death due to septicaemia occurring after the gallbladder rupture. 
P. mirabilis, P. vulgaris, P. cibarius, and P. penneri were identified in all the samples, except for the pus collected from the kidneys. However, their pathogenicity in animals has not been reported.

Escherichia fergusonii, a Gram-negative opportunistic pathogen causing UTIs was identified in the kidneys (Lai et al. 2011; Glover et al. 2017). However, this screening PCR test ruled out the most common organisms known to cause biliary tract infections such as E. coli and Klebsiella pneumoniae. Although Proteus species are saprophytes and saprogenic bacteria reside in soil, sewage, and carcass, it was confirmed that the infection occurred when the bird was alive, and death resulted from a Proteus infection as the penguin’s body was maintained at 4 °C during the transportation and the necropsy was performed within 12 h of death. Moreover, the presence of the bacterial colonies in various organs including the parenchymal organs such as the liver, the DIC in the blood vessels, and the secondary inflammatory responses, such as infiltration of the chronic active inflammatory cells in various organs, were observed microscopically. 
Proteus, a proteobacterium belonging to the family Enterobacteriaceae, is a well-known opportunistic pathogen mainly reserved in the intestines of humans as well as domestic and wild

DISCUSSION AND CONCLUSIONS

Septicaemia is a life-threatening and complicated form of bacteraemia characterised by fever, toxae mia, organ dysfunction, and final shock (Zachary and McGavin 2011; Tizard 2012). It is indicated that one of the most common aetiologies for septicaemia is a biliary tract infection, which is most commonly caused by the family Enterobacteriaceae ascending from the gastrointestinal tract (Melzer et al. 2007). The bacteria while ascending invades the biliary tract from the duodenum (Sung et al. 1992). The increased intraductal pressure leads to biliary content reflux and bacteraemia, which, in turn, causes septic shock and, finally, sudden death (Hanau and Steigbigel 2000).

In the present case, the penguin showed several lesions representing a systemic bacterial infection on both the H&E and Gram staining. For instance, several parenchymal organs showed infiltration of the chronic active inflammatory cells, detachment of the epithelial cells, haemorrhages, and congestion. Particularly, the bile ducts were infiltrated by the inflammatory cells with haemorrhaging; microscopically, bile pigments were also observed
animals (Rozalski et al. 1997; Drzewiecka 2016). These bacteria can cause severe pathologic conditions in humans and animals with an immunocompromised status with an environmental stress, a co-morbid disease, or a concurrent infection (Sah et al. 1983; Rozalski et al. 1997; Olinda et al. 2012). Proteus septicaemia in avian species has been clinically characterised by rapid depression, coma, and sudden death and pathologically by severe congestion of the parenchymal organs such as the liver, lungs, and kidneys (Sah et al. 1983). Similarly, in the present case, the penguin exhibited an abrupt and rapid depression and coma before death. Moreover, most of the parenchymal organs, such as the liver, kidneys, and lungs showed severe congestion and haemorrhaging. However, the most interesting gross lesion in this present case was the gallbladder rupture accompanied by a secondary bilious effusion in the whole abdominal cavity. In addition, a purulent exudate was observed in the other organs such as the tracheal carina, lungs, and kidneys. 

Humboldt penguins live in colonies (Simeone et al. 2004). However, in the present case, the penguin was isolated and introduced to a new rearing aquarium during the season of moulting because it ruthlessly attacked other penguins in the zoo flock. A biliary infection, which shows high morbidity and mortality, is associated with immunosuppressed patients exposed to stress, a primary underlying disease, a co-morbid disease, and a co-infection by other bacterial pathogens (Melzer et al. 2007). Therefore, in this case, the possibility of stress associated with the environmental changes cannot be excluded as a contributing factor along with the Proteus infection, and it also explains the concurrent infection with another Gram-negative opportunistic pathogen, E. fergusonii. Thus, Proteus residing in the intestines retrograded via the small intestine. Sequentially, the ascending infection toward the gallbladder may have resulted in cholecystitis, which, in turn, led to a blood flow obstruction of the gallbladder, leading to necrosis and the gallbladder rupture. Bile ascites that leaked from the gallbladder probably caused bile peritonitis, resulting in systemic inflammatory response syndrome (SIRS). For these reasons, it is highly suspected that the penguin died due to septic shock.

A case of septicaemia caused by Proteus species in a bird was reported (Sah et al. 1983). Although Proteus is known as a potential pathogen in birds (Olinda et al. 2012), it is an opportunistic pathogen that does not cause diseases in healthy animals. For this reason, neither septicaemia due to Proteus infections nor the pathological examination in penguins have been reported, although few cases of septicaemia caused by other pathogens, such as P. aeruginosa and Erysipelothrix rhusiopathiae, in penguins were reported (Boerner et al. 2004; Widmer et al. 2016). In addition, S. humboldti has been categorised as a threatened species, therefore, we can mostly see these penguins in zoos, but pathological information of septicaemia in this species has been rarely reported; thus, the detailed histopathological description of septicaemia caused by the bacterium may provide a clue for the diagnosis of septicaemia in penguins caused by Proteus and other bacterial species later on. For this reason, we hereby report this apparently first known case of septicaemia due to Proteus infection in a Humboldt penguin.

REFERENCES


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